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13. ABSTRACT *(Maximum 200 Words)*

We have generated a panel of 100 anti-Her-2 MAbs and have characterized them with regard to isotype, epitope recognition and ability to signal apoptosis in Her-2-overexpressing breast cancer cell lines. Twelve of these MAbs, recognizing nine different epitopes on the Her-2 molecule, negatively signal Her-2-overexpressing tumor cells. In parallel work which we are carrying out using MAbs against human lymphoma cells, we have observed that chemically prepared tumor-reactive MAb homodimers (IgG-IgG) of MAbs induce significantly more growth arrest and death than their monomeric (IgG) counterparts (11), probably because of hypercrosslinking (12). In our original application, we proposed evaluating the antitumor activity of our best 10 anti-Her-2 dimers. In the first six months of the project, we prepared IgG homodimers of representative MAbs recognizing the nine different epitopes on the Her-2 molecule. These homodimers were evaluated for their ability to induce apoptosis in Her-2-overexpressing human breast cancer cell lines. Based on these studies we choose three MAbs for follow-up studies. At this time, we have designed and are in the process of expressing three different recombinant homodimeric constructs with the Fv regions of these three MAbs.

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INTRODUCTION

The aim of this proposal (funded in 1999) was to determine whether IgG homodimers or $F(ab')_2$ fragments of homodimers of our highest affinity anti-Her 2 monoclonal antibodies (MAbs) alone, or in combination with chemotherapy would make potent anti-tumor agents in SCID mice engrafted with Her-2^+ human breast cancer cell lines. Homodimers were first prepared and tested *in vitro* and then evaluated in our newly-developed SCID xenograft model of Her-2^+ breast cancer cell lines. Homodimers will now be evaluated for pharmacokinetics, biodistribution and anti-tumor activity in both subcutaneous tumors and in disseminated disease. The most effective homodimers will be cloned, expressed, purified and tested as recombinant therapeutic molecules.

BODY:

Task 1: **To prepare and test homodimers *in vitro*.** This aim was completed and described in the 1999 progress report.

Task 2: **To continue characterization of the SCID xenograft mice including growth pattern, PCR detection, end points, etc.** This has been accomplished and is described in the accepted publication by Clinchy, B., Rabinovsky, R., Gazdar, A., Yefenof, E., Gordon, B. and Vitetta, E.S. The growth and metastasis of human, HER-2/neu-overexpressing tumor cell lines in male SCID mice. **Breast Cancer Research**, In press, 2000. (See appendix).

Task 3: **To carry out single and "cocktail" testing of homodimers and monomers in three breast cancer cell lines.** The single agents were tested and described in our 1999 Progress Report. To date, we have evaluated the individual homodimers and monomers *in vitro* but have not yet completed the cocktail testing. We are testing them ± cisplatin. The representative results shown in Figure 1 indicate that the homodimers 10-100 fold better than the monomers in inducing death in the breast cancer lines *in vitro*, both in the presence and absence of cisplatin.

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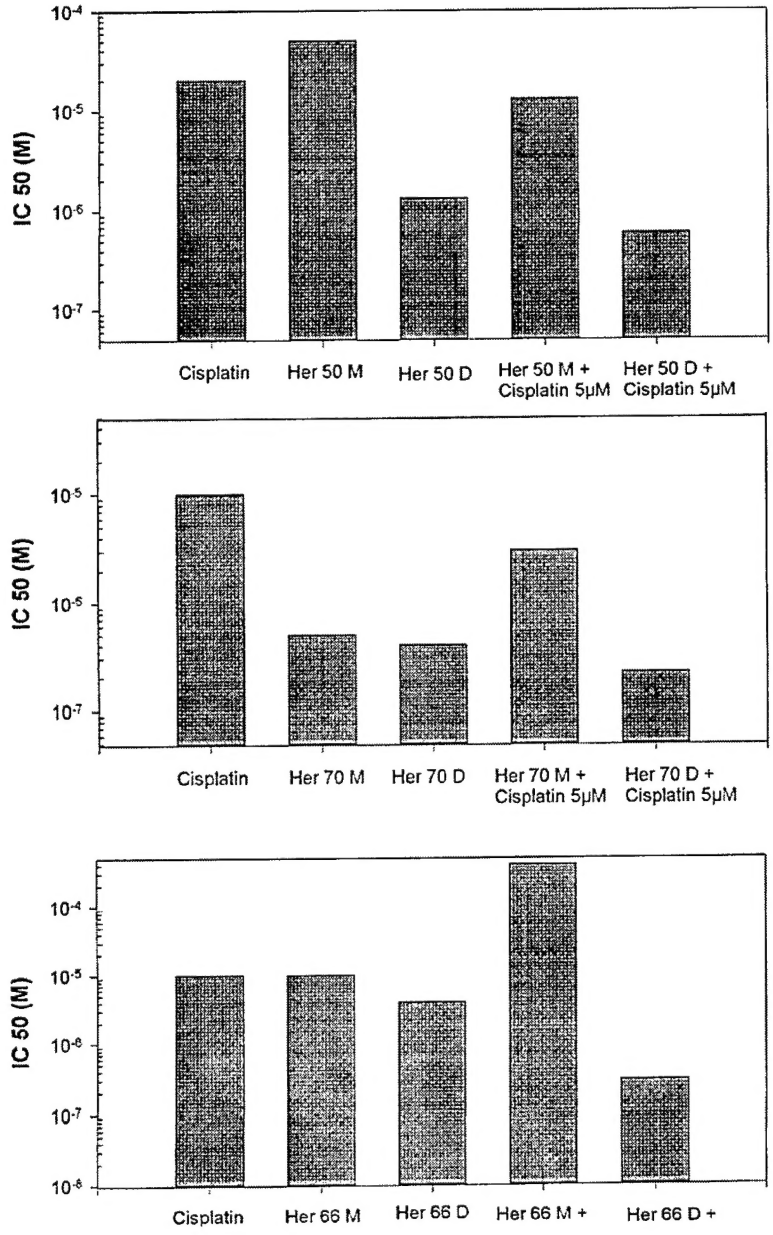

Figure 1. Cytotoxic activity of HER 50, HER 66, and HER 70 Monomers and Homodimers ± 5 µM Cisplatin. The anti-proliferative activities of the HER-50 (top panel), HER70 (middle panel), and HER66 (lower panel) of monomers (M) and homodimers (D) were tested on BT474 cells. BT474 cells [2.5 x 10^5 cells/100 µl in MEM containing 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 nM non-essential amino acids, 1 nM sodium pyruvate and 2% vitamins for MEM] were plated into triplicate wells of 96 well microtiter plates and allowed to adhere overnight. The cells were then treated with 100 µl of anti-Her-2 MAbs diluted in the same medium. The plates were incubated for 72 hours at 37°C in 5% CO_2 and pulsed for 6 hours with 1 µCi [^{3}H]-thymidine. Nine wells of untreated cells were included in each experimental plate as controls. This is a representative experiments of 7 carried out. The data represent the IC_{50} values of the concentrations of MAbs or cisplatin necessary to kill 50% of cells

Task 4: To determine the LD_{50} of the anti-tumor activity of tamoxifen, taxol and cisplatin in SCID mice carrying three different tumors. The LD_{50}'s are shown below:

	LD_{50} (mg/kg)	
Drug*	**i.p.**	**i.v.**
Taxol	128	12
Cisplatin	6.6	11

*Tamoxifen must be tested

Task 5: To determine the biodistribution and pharmacokinetics of monomers and homodimers in SCID/BT474 xenografted mice. Based on the results of the task described above one of the three monomers was first injected into SCID mice and its biodistribution evaluated. As shown in Figure 2, in the case of HER-66, in mice with subcutaneous tumors, there is a considerable amount of material taken up by the subcutaneous breast tumor nodule. The testing of the other two homodimers is in progress.

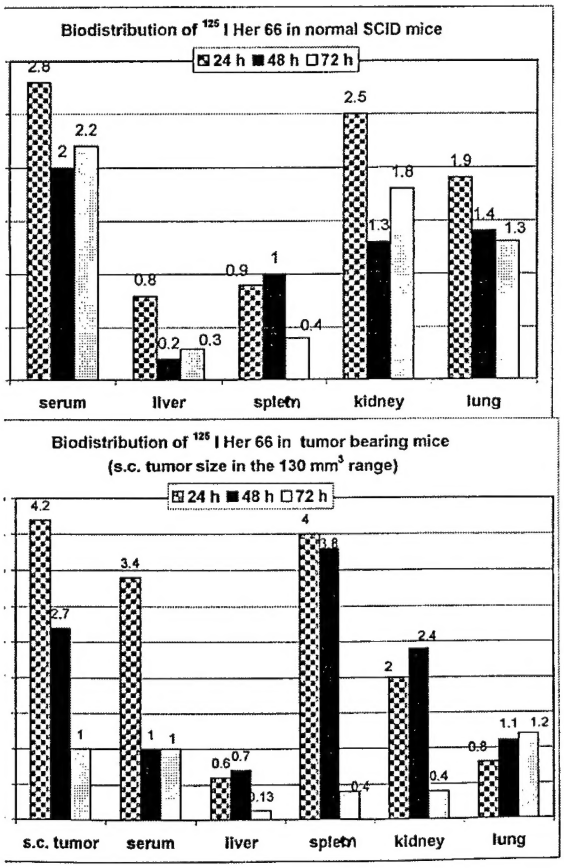

Figure 2. Biodistribution of ^{126}I HER 66 in A) normal SCID mice. B) SCID mice with 130mm^3 subcutaneous BT474 tumors. Radiolabeled anti-Her2 MAb (HER66) was injected i.p. into 2 groups of 3 mice. Mice were sacrificed at 27, 48 and 72 hours. Perfused organs were weighed. The radioactivity of each organ as well as tumor and blood were measured. Results are expressed as % of injected dose per gram of tissue (%ID/g).

Task 6: Based on the results of Task 4, set up *in vivo* therapy experiments in early and late tumor. We have approached this aim by first carrying out experiments in mice with subcutaneous tumors. We did this in order to determine whether homodimers would reach the tumor and shrink these "easy-to-measure" subcutaneous nodules. As shown in Figure 3 the HER50 homodimers do indeed reach the tumor site and induce tumor reduction. (HER70 and 66 are now being evaluated). However, tumors begin to grow again after approximately a week. We are in the process of determining whether the tumors which grow back lack the Her-2 epitope targeted by HER50. Nevertheless, since we do not intend to use these molecules in bulky disease the experiments suffice to tell us that the homodimers can have a therapeutic effect even in these solid tumors. We are now in the process of setting up the disseminated tumor model (Clinchy, et al, Breast Cancer Research, in press) where the model is a more realistic approximation of the situation in humans.

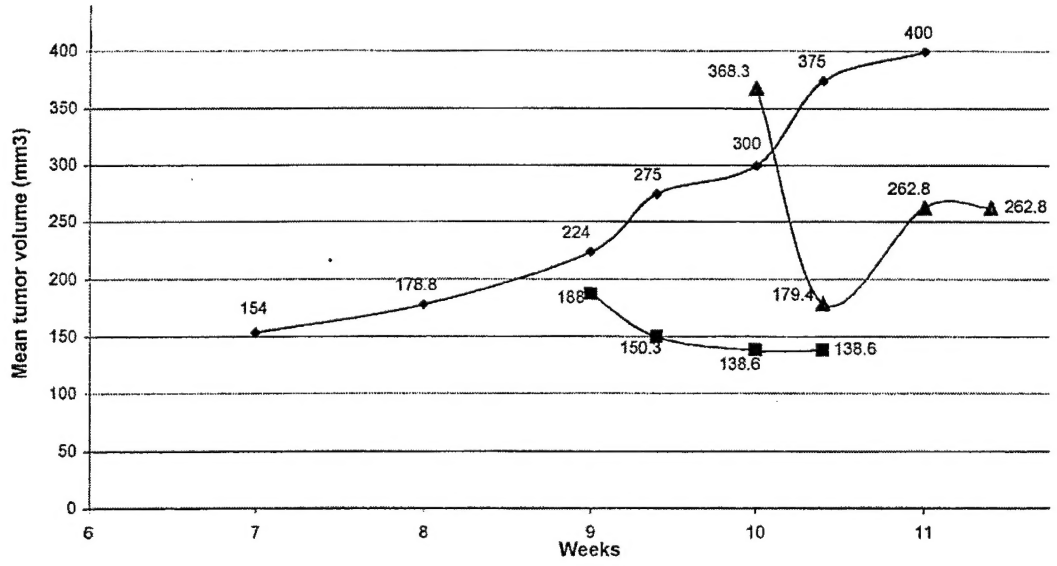

Figure 3. Subcutaneous BT474 tumors in mice following treatment with HER50 homodimers. Mice were inoculated with BT474 tumor and were treated with MAbs. The mean tumor volume was measured at different intervals. ● 25 untreated SCID/BT474 mice ($2.5x10^6$ cells/mouse, s.c.), ■ 5 SCID/BT474 mice injected IP with 1 mg HER 50 homodimers (2 doses, 500 μg/dose), ▲ second group of 5 mice, same treatment

Task 7: Begin cDNA cloning of the best hybridomas. We are cloning the V regions of the HER66, 70 and 50. We intend to insert these V regions into a human IgG_1 scaffold to make chimerized monoclonal antibodies. To this we have engineered a plasmid carrying the human IgG_1 and introduced a cystine at position 444 at the C terminal of the H chain. Expression levels are ~ 5 mg/liter (Figure 4).

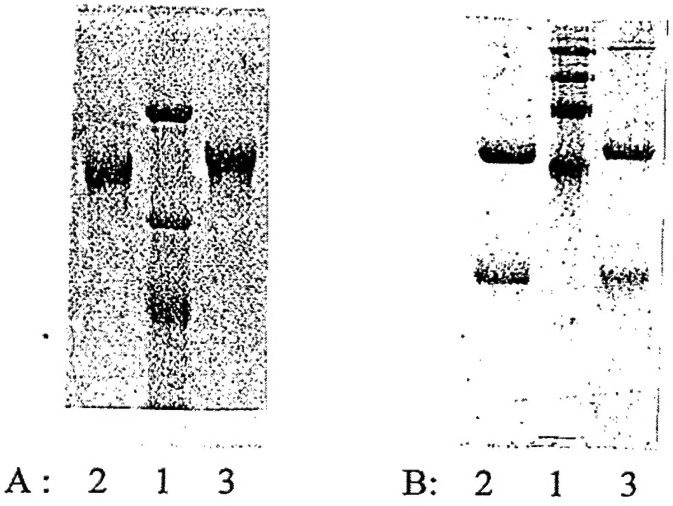

Figure 4. The expression of Human IgG-mouse Fv chimeric MAb (another specificity) was analyzed by SDS-Page (the human H chain has a cysteine in position 444). The culture supernatants were purified on R anti Hu IgG affinity columns. Purified material was electrophoresed on both reducing and nonreducing gels. (A) nonreducing gel, (B) reducing gel. Lane 1: protein marker, (205K, 121K, 74K, 477K); Lane 2: Human IgG; Lane 3: HuIgG-444-mouse Fv.

When these molecules are expressed they can be then treated with Elman's reagents to generate homodimers with excessable binding regions (1).

Tasks 8 and 9: These have not yet begun and will take place over the next year.

KEY RESEARCH AND ACCOMPLISHMENTS:

A SCID xenograft model has been set up and three homodimers are being evaluated for biodistribution, pharmacokinetics, and anti-tumor activity. A strategy to generate chimeric (human/mouse) IgG antibodies has been developed and the Fvs of HER66, 50 and 70 are being cloned. They will then be inserted into the human IgG_1 plasmid. When we submitted this grant we were unclear as to which recombinant constructs we would use, but we now feel that there is compelling evidence to prepare a chimeric $HIgG_1$ dimer.

REPORTABLE OUTCOMES:

Clinchy, B., Rabinovsky, R., Gazdar, A., Yefenof, E., Gordon, B. and Vitetta, E.S. The growth and metastasis of human, HER-2/neu-overexpressing tumor cell lines in male SCID mice. **Breast Cancer Research**, In press, 2000.

CONCLUSIONS:

We have shown the homodimers (± chemotherapy) have greater anti-tumor activity *in vitro* than their corresponding monomers. We have set up a murine xenograft model for disseminated Her-2^+ human breast carcinoma and have characterized the model thoroughly. We have demonstrated that one of the homodimers has good biodistribution and some anti-tumor activity in subcutaneous disease. We have selected a strategy for generating recombinant antibodies and are using the three optimal anti-Her-2 MAbs to prepare recombinant homodimers. In the next year we plan to test the chemically prepared homodimers (± chemotherapy) in the disseminated breast tumor model. At the same time we will be preparing the chimeric recombinant molecules to test *in vitro*. We hope to initiate the *in vivo* experiments before the grant expires.

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